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Diarylspiro[2.4]heptenes as selective cyclooxygenase-2 inhibitors: A quantitative structure-activity relationship analysis

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Both the Fujita-Ban and Hansch quantitative structure-activity relationship (QSAR) analyses, attempted on the data set of 5,6-diarylspiro[2.4]hept-5-enes as the inhibitors of cyclooxygenase-2 (COX-2) have helped to ascertain the role of R- and X-substituents in explaining their observed biological inhibition actions. From both approaches it is concluded that the substitution NH_2 instead of Me at R is desirable. In addition, the 3-F and/or 5-Cl having smaller molar refraction values and the 4-Cl and 4-CF_3 possessing less hydrophobic-cum-hydrogen acceptor property at X are the preferred substitutions of the aryl ring. For those analogues whose COX-1 inhibition data are available, the selectivity ratio, -logS [S = IC_{50}(COX-2)/IC_{50}(COX-1)], is found to correlate with the electronic and hydrophobic-cum-hydrogen acceptor parameters. From the derived significant correlation, it follows that the higher electron-withdrawing effect produced by 3- and 4-X and the lower hydrophobic-cum-hydrogen accepting effect by 4-X in the aryl ring are highly beneficial.

The chronic inflammatory conditions of arthritic patients may be checked[1] by nonsteroidal antiinflammatory drugs (NSAIDs) such as aspirin. These drugs possess antiinflammatory, analgesic and antipyretic activities but are not always completely devoid of life-threatening side effects, namely gastrointestinal haemorrhage, ulceration[1,2] and decreased renal function in some cases[3,4]. In view of such problems, efforts are being made to have new NSAIDs that possess antiinflammatory activity without the toxic side effects.

Huang *et al.*[5] have recently reported 5,6-diarylspiro[2.4]hept-5-enes as orally active and highly selective COX-2 inhibitors. These compounds (Figure 1) have been shown to possess promising pharmacological properties in adjuvant-induced arthritis and edema analgesia models.

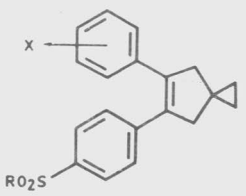

Fig. 1—Structures of 5,6-diarylspiro[2,4]hept-5-enes

With a view to deriving quantitative structure-activity relationships (QSARs), we report here the Fujita-Ban analysis as well as the Hansch analysis using the reported biological activities and physicochemical parameters[6] of the substituents.

Methodology

First and foremost among the QSAR methods is the model proposed by Hansch and co-workers[6-11]. It was the seminal contribution of this group to propose that the molar concentration (or dose) that elicits a constant biological response of a drug molecule is the linear function of its physicochemical parameters governing various types of interactions. The lipophilicity, π, the electronic, σ, the Taft's steric, Es, the molar refraction, MR etc are most common parameters of their formalism. Multiple regression analysis[12-14] (MRA) is used to derive the best QSARs. The resulting correlations are assessed through a number of statistics obtained in conjunction with such calculations. The most important of these are the standard error of the estimates, s, the correlation coefficient, r (or the multiple correlation coefficient, R), the F-ratio, a statistic for assessing the overall significance of the derived equation and the confidence intervals

(usually 95%) for the individual regression coefficients in the equation.

The observation that in an analogue series the substituents ought to contribute constant increments or decrements to biological activity, BA led Free and Wilson[15] to develop an extrathermodynamic QSAR approach, which is based on an additivity principle as given by Eq. (1)

$$BA_i = \Sigma a_j X_{ij} + \mu \quad \ldots(1)$$

Where X_j is the jth substituent with a value of 1 if present and 0 if not, a_j is the contribution of the jth substituent to BA, and μ is the overall average activity. All activity contributions at each position of substitution must sum to zero. The series of linear equations so generated is solved by the method of least squares for a_j and μ. Fujita and Ban[16] later modified this method in which symmetry equations are not required and μ was interpreted as the theoretical biological activity value of the unsubstituted reference compound of a series. This definition then resembles more closely to the Hammett's linear free energy relationship (LFER), because the activity contributions a_j now refer to hydrogen in the same position of substitution.

The compounds under study, their biological effects alongwith various substitutional parameters are listed in Table I. The activity data IC_{50} represents the concentration of a drug to accomplish 50% inhibition of the human COX-2. The same are expressed as $-\log IC_{50}$ on molar basis. In addition, the COX-1 activities are reported only for a few compounds. For these analogues, the selectivity ratio, $S = IC_{50}(COX\text{-}2)/IC_{50}(COX\text{-}1)$, computed as -logS are also included in Table I.

Results and Discussion

Table I shows that in the aryl ring at 4-position the substituent X = F, Cl, OMe etc are present in all the compounds and none has X = H. Compound

Table I-QSAR parameters for 5,6-diarylspiro[2.4]hept-5-enes as highly selective cyclooxygenase inhibitors (Figure 1 for structures)

Compd	X	R	$MR_{3.5}$	$\sigma_{3.4.5}$	π_4	HA_4	I_4	I_R	$-\log IC_{50}(M)$[a]			$-\log S$[c]	
									Obsd	Calc Eq.4	Calc F.B.	Obsd	Calc Eq.7
1	4-F	Me	0.206	0.06	0.14	0	1	0	8.10	8.10	8.29	2.83	2.71
2	4-F	NH_2	0.206	0.06	0.14	0	1	1	8.52	8.55	8.73	2.04	1.68
3	4-Cl	Me	0.206	0.23	0.71	0	0	0	9.00	8.75	8.74	3.49	3.42
4	4-Cl	NH_2	0.206	0.23	0.71	0	0	1	9.00	9.20	9.18	2.15	2.40
5	4-OMe	Me	0.206	-0.27	-0.02	1	0	0	8.30	8.34	8.27	2.06	2.37
6	4-OMe	NH_2	0.206	-0.27	-0.02	1	0	1	9.00	8.79	8.71	0.70	1.35
7	4-OCF_3	Me	0.206	0.35	1.04	1	0	0	6.87	6.65	6.66	UA	-
8	4-OCF_3	NH_2	0.206	0.35	1.04	1	0	1	6.89	7.20	7.10	1.14	1.17
9	4-CF_3	Me	0.206	0.54	0.88	0	0	0	8.70	8.49	8.63	UA	-
10	4-CF_3	NH_2	0.206	0.54	0.88	0	0	1	9.00	8.94	9.07	2.70	2.69
11	3-F,4-OMe	Me	0.195	0.07	-0.02	1	0	0	8.00	8.35	8.20	UA	-
12	3-F,4-OMe	NH_2	0.195	0.07	-0.02	1	0	1	8.70	8.80	8.63	2.08	1.87
13	3-Cl,4-OMe	Me	0.706	0.10	-0.02	1	0	0	7.77	8.20	8.11	UA	-
14	3-Cl,4-OMe	NH_2	0.706	0.10	-0.02	1	0	1	8.70	8.65	8.55	3.15	-[d]
15	3-Br,4-OMe	Me	0.991	0.12	-0.02	1	0	0	7.89	8.12	8.08	UA	-
16	3-Br,4-OMe	NH_2	0.991	0.12	-0.02	1	0	1	8.70	8.57	8.51	2.30	1.94
17	3,4-F_2	Me	0.195	0.40	0.14	0	1	0	8.48	8.11	8.22	UA	-
18	3,4-F_2	NH_2	0.195	0.40	0.14	0	1	1	8.52	8.56	8.65	2.49	2.20
19	3,4-Cl_2	Me	0.706	0.60	0.71	0	0	0	8.52	8.61	8.58	UA	-
20	3,4-Cl_2	NH_2	0.706	0.60	0.71	0	0	1	9.00	9.06	9.02	2.58	2.96
21	3-Cl,4-F	Me	0.706	0.43	0.14	0	1	0	8.16	7.96	8.14	2.93	3.27
22	3-Cl,4-F	NH_2	0.706	0.43	0.14	0	1	1	8.82	8.41	8.57	2.32	2.25
23	3,5-Cl_2, 4-OMe	Me	1.206	0.47	-0.02	1	0	0	8.22	8.06	8.09	UA	-
24	3,5-Cl_2, 4-OMe	NH_2	1.206	0.47	-0.02	1	0	1	8.40	8.51	8.53	3.61	-[d]
25	4-Me	Me	0.206	-0.17	0.56	0	0	0	8.82	8.97	-[b]	3.43	2.97

[a]The IC_{50} represents the concentration of a compound for 50% inhibition of the enzyme COX-2; taken from ref. 5.
[b]Omitted compound in Fujita-Ban (F.B.) study.
[c]Selectivity ratio, $S = IC_{50}(COX\text{-}2)/IC_{50}(COX\text{-}1)$ and UA is the reported uncertain activity value for the COX-1 (ref. 5).
[d]The 'outlier' compounds in obtaining Eq. (7).

1 with X = 4-F and R = Me has, therefore, retained as the parent compound for calculating μ. Compound **25** of this table is the lone compound which has X = 4-Me. The same was ignored in constructing the Fujita-Ban matrix, while COX-2 activities being as the dependent variable. The matrix (omitted for the sake of brevity) has resulted into 24 linear equations, each in 10 independent variables including parent contribution μ. These were then solved by the method of MRA and the results are given in Table II. The derived statistical parameters, R, s and F are in tune with high level of significance of these results as the F-ratio is significant at 99% level [$F_{10,13}(0.01) = 4.10$] and R^2-value accounts for 90% of the variance. From the calculated values of substituents contributions (Table II), it follows that the substitution NH_2 instead of Me at R is desirable as it has a positive contribution (= 0.437) relative to Me. The sum of this contribution and the contribution of parent compound (= 8.293) leads to enhanced value of the $-\log IC_{50}$ (= 8.730). In addition, the X-substitution in aryl ring such as 4-Cl or 4-CF_3, with their contribution of 0.448 and 0.339 respectively, further improves the inhibitory activity of a compound, but none of the substituents seems to be appropriate for 3- and 5-position of this aryl ring. For steric reasons, these meta-positions better remain unsubstituted. However, 3-F and/or 5-Cl, which are having least negative contributions in aryl ring may be the alternative selections in future synthetic efforts. The calculated values of $-\log IC_{50}$ obtained by adding the requisite substituents contribution to μ, are in close agreement with the observed ones (Table I). These findings are further corroborated in the light of undermentioned Hansch type of study.

Table II- Fujita-Ban substituent contributions of 5,6-diaryl-spiro[2.4] hept-5-enes to inhibition action against the enzyme COX-2

Parent Contribution, μ = 8.293(±0.143)

Position	Substituent Contributions
3-X	F=–0.075(±0.168), Cl=–0.158(±0.143), Br=–0.195(±0.221)
4-X	Cl=0.448(±0.168), OMe=–0.022(±0.143), OCF_3=–1.631(±0.221), CF_3=0.339(±0.221)
5-X	Cl=–0.021(±0.221)
R	NH_2=0.437(±0.101)

n=24, R=0.949, s=0.248, F(10,13)=11.690

Multiple regression analysis of the data for above set of compounds (data points, n = 24), yielded regression Eq. (2) for the COX-2 inhibition activity versus their different physico-chemical parameters.

$$-\log IC_{50} = -0.207(\pm 0.145)MR_{3,5} - 1.472(\pm 0.171)\pi_4 - 1.604(\pm 0.146)HA_4 - 1.360(\pm 0.166)I_4 + 0.437(\pm 0.089)I_R + 9.858$$

n=24, R=0.945, s=0.219, F(5,18)=29.783 ...(2)

The ± data within parentheses are the 95% confidence limits. The variable $MR_{3,5}$ represents the sum of molar refractions of the meta-substituents, X on the aryl moiety. The hydrophobic contribution and hydrogen acceptor property of *para*-substituents on this ring are represented, respectively by π_4 and HA_4. Two dummy variables, I_4 and I_R were chosen for the subscripted positions so as to account for the presence or absence of certain structural features thereon. For example, an NH_2 substitution at R is indicated by 1, while a Me is assigned the value 0. Analogously, I_4 is taken as 1 when X = 4-F and 0 otherwise. A large number of correlations in other parameters such as electronic σ, steric Es, Field F, resonance R, van der Waals volume Vw etc were attempted for X-substitution in various possible combinations, but none of them could yield better results than that of Eq. (2). The F-value of above equation is significant at 99% level [$F_{5,18}(0.01)$= 4.25] and R^2 takes care of 89% of the variance in the observed activity values. Though these statistical parameters are hinting at the high level of significance of Eq. (2) but the inter-correlation matrix (not given here), obtained among independent variables of this equation, has shown that the orthogonality condition is not satis- fied between HA_4 and I_4 (r=0.58) variables. Also $MR_{3,5}$ versus π_4 and π_4 versus HA_4 correlations having slightly higher R-values (= 0.38 in each) are not truly independent. Eq. (2) as such may, therefore, mislead the QSAR results. In order to improve its significance further, the hydrophobic parameter π_4, the hydrogen acceptor paremeter HA_4 and the fluorine preferential parameter I_4 were added together and their sum is denoted by a new parameter, β_4. The combination of these variables is a result of the facts that the calculated regression cofficients of these are all negative (Eq. 2), weigh nearly equal in magnitude and have the comparable ± confidence intervals. It is evident

from Eq. (2) that the presence of either a highly electronegative substituent, 4-F or a hydrogen-acceptor substituent, 4-OMe in aryl ring reduces the activity value (negative correlation coefficient of I_4 and HA_4 respectively), while their low hydrophobic character would increase it. These two causes, therefore, operate in opposite directions and the net gain in inhibiton action would depend on the resultant value, expressed as β_4. Generally, the increase in activity with the decrease in hydrophobic property is an indicative of some kind of polar interaction. However, the attempted polar parameters such as the field, F or resonance, R could not replace the β_4 parameter in the follow-up correlation equations. Thus employing the β_4 along with $MR_{3,5}$ and I_R variables in the MRA, the correlation Eq. (3) was obtained.

$$-\log IC_{50} = -0.302(\pm 0.139)MR_{3,5} - 1.535(\pm 0.149)\beta_4 + 0.437(\pm 0.095)I_R + 9.931$$

$$n=24,\ R=0.930,\ s=0.233,\ F(3,20)=42.598 \qquad \ldots(3)$$

And consideration of all 25 data points (including compound 25) resulted into slightly improved correlation Eq. (4)

$$-\log IC_{50} = -0.281(\pm 0.134)MR_{3,5} - 1.500(\pm 0.139)\beta_4 + 0.449(\pm 0.092)I_R + 9.872$$

$$n=25,\ R=0.930,\ s=0.230,\ F(3,21)=44.514 \qquad \ldots(4)$$

Both these trivariant equations are highly sound in statistical parlance and their results are superior to that of a five variable Eq. (2). The F-value of Eq. (3) and Eq. (4) are significant at 99% level [$F_{3,20}(0.01)= 4.94$; $F_{3,21}(0.01)= 4.87$] and their R^2-values account for 84% of the variance. All the variables of Eq. (4), which is derived from complete data set, are mutually independent (r: $MR_{3,5}$ vs $\beta_4 = 0.162$; $MR_{3,5}$ vs $I_R = 0.030$ and β_4 vs $I_R = 0.060$). It also follows from Eq. (4) that NH_2 instead of Me at R would lead to better inhibitors. A large negative coefficient of β_4, *inter alia*, denotes that a less hydrophobic-cum-hydrogen acceptor substitution on *para*-position in the phenyl ring would be beneficial. To this effect, a hydrogen acceptor substitution such as 4-SO_2NH_2 with a low π_4 value (= -1.82) seems to be the most appropriate. Thus a compound with X = 4-SO_2NH_2 and R = NH_2 is found to have the highest theoretical activity value ($-\log IC_{50}$ = 11.50, Eq. 4). This compound is not described in the original reference and should be synthesised and screened for experimental activity value. In addition, the *meta*-substituents in same phenyl ring having smaller molar refraction value (a measure of steric bulk) are desirable. In order to achieve this, both of these positions better remain unsubstituted. The smaller hydrogens at them may not sterically hinder the orientation of a compound at the receptor site during its action. Using Eq. (4), the calculated values of $-\log IC_{50}$, listed in Table I, are highly in agreement with the observed ones.

For inhibition of the enzyme COX-1, only a few compounds are reported active. Eight compounds having uncertain activity (UA) values for this enzyme are ignored in further study. The resulting correlation between $-\log IC_{50}$'s of the COX-1 and the COX-2 is shown in Eq. (5)

$$-\log IC_{50}(\text{COX-1}) = 0.877 - \log IC_{50}(\text{COX-2})$$

$$n=17,\ r=0.394,\ s=0.768,\ F(1,15)=2.762 \qquad \ldots(5)$$

Obviously, it has emerged as a poor correlation, which suggests that inhibition actions of the title compounds are not the same at the COX-1 and the COX-2. Alternatively, it may be stated that the two enzymes function in different ways and the mode of interaction of a compound at these enzymes will also be different. In view of this fact, a large number of physicochemical parameters were correlated with selectivity ratio, -logS and one of the best correlation that was emerged is given in Eq. (6).

$$-\log S = 1.576(\pm 0.535)\sigma_{3,4,5} - 1.174(\pm 0.434)\beta_4 - 0.820(\pm 0.312)I_4 + 3.923$$

$$n=17,\ R=0.790,\ s=0.540,\ F(3,13)=6.746 \qquad \ldots(6)$$

Ignoring two compounds **14** and **24**, which followed off-the-trend behaviour, a regression Eq. (7) showing large improvement over Eq. (6) was obtained.

$$-\log S = 1.520(\pm 0.375)\sigma_{3,4} - 1.056(\pm 0.294)\beta_4 - 1.024(\pm 0.217)I_R + 3.819$$

$$n=15,\ R=0.900,\ s=0.365,\ F(3,11)=15.659 \qquad \ldots(7)$$

In Eq. (6), the variable $\sigma_{3,4,5}$ represents the summation of electronic parameters of various substituents present at 3-, 4- and 5-positions in the aryl ring. This variable is expressed as $\sigma_{3,4}$ (the summed value of 3- and 4-substitutions) in Eq. (7) as compound **24** being the only compound which has a 5-Cl substitution and was omitted in deriving

this equation. The 'outlier' behaviour of this compound may be attributed to its being loneliness in the series. However, no immediate reason is apparent for indifferent behaviour of compound **14**. The F-value of Eq. (7) is significant at 99% level [$F_{3,11}(0.01)=6.22$] and R^2-value accounts for 81% of the variance. Further, this equation has indicated that a Me- instead of NH_2- substitution at R leads to higher selectivity ratio. Likewise, in second aryl ring the *para*-substitution having lower hydrophobic- cum-hydrogen acceptor property and *meta*- and *para*-substitutions collectively producing high electron-withdrawing effect are desirable. Using Eq. (7), the calculated –logS values (Table I) closely resemble the observed ones and all variables on the right hand side of this equation are poorly inter-correlated (r: $\sigma_{3,4}$ vs $\beta_4=0.17$; $\sigma_{3,4}$ vs $I_R=0.35$ and β_4 vs $I_R=0.23$).

In conclusion, it may be stated that the present QSAR study provides a rationale for the design of cyclooxygenase inhibitors, and a basis for substituent-selection in drug-design strategy. It also helps in deciphering the drug-receptor interaction at the molecular level.

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